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# FACSIMILE TRANSMISSION COVER SHEET

TO: **Honorable Commissioner of Patents and Trademarks**
**Attn: June Hwu**
**Washington, DC 20231**

PTO FACSIMILE NUMBER: (703) 872-9306

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COMMENTS: **Re: U.S. Serial No. : 09/600,500**
**J&J Docket No.: J&J 1763**

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(732) 524-2818

Docket No. J&J-1763

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

| | | | |
|---|---|---|---|
| Applicants | : | Anthony J. Fist et al. | |
| Serial No. | : | 09/600,500 | Art Unit: 1638 |
| Filed | : | July 14, 2000 | Examiner: June Hwu |
| For | : | IMPROVED PRODUCTION OF RETICULINE | |

I hereby certify that this correspondence is being faxed to the Assistant Commissioner for Patents, Washington, D.C. 20231. Facsimile Number: 703-872-9306

March 22, 2004
(Date)

John W. Harbour
Name of applicant, assignee, or Registered Representative

(Signature)

March 22, 2004
(Date of Signature)

Honorable Commissioner of Patents
Washington, D.C. 20231

Dear Sir;

**Submission of Replacement Pages**

In response to a phone call from Examiner June Hwu, applicants attach replacement pages 9-10 and 23-25 of the specification, which the Examiner reported missing from the official file history.

Applicants attach a copy of the postcard receipt for the filing of the application on July 15, 2000, which was received by applicant from the PTO on July 26, 2000. This postcard reflects that 32 pages of the specification were included with the filing. Thus, applicants submit that the attached pages are not new matter and are simply replacement pages.

Respectfully Submitted

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Preferably steps a) to c) are repeated until the (S)-reticuline content shows no further increase on mutagenesis.

According to a thirteenth aspect there is provided a method for the production of (S)-reticuline which comprises the steps of:

a) harvesting poppy capsules of a stably reproducing *Papaver somniferum* to produce a straw wherein the straw has an (S)-reticuline to phenanthrene alkaloid ratio of 100% or greater by weight, and

b) chemically extracting the (S)-reticuline from the straw.

According to a fourteenth aspect there is provided a method for the production of (S)-reticuline which comprises the steps of:

a) collecting and drying the latex of the immature poppy capsules of a stably reproducing *Papaver somniferum* to produce opium wherein the opium has an (S)-reticuline to phenanthrene alkaloid ratio of 100% or greater by weight, and

b) chemically extracting the (S)-reticuline from the opium.

According to a fifteenth aspect there is provided a method to improve the (S)-reticuline yield of a stably reproducing *Papaver somniferum*, the method comprising the steps of:

a) exposing at least one poppy seed of *Papaver somniferum* to a mutagenizing agent,

b) growing the at least one poppy seed to produce a plant bearing a leaf or an immature poppy capsule, optionally through multiple self fertilized generations,

c) sampling the leaf or poppy capsule for the presence of (S)-reticuline, morphine and codeine, and

d) repeating steps a) to c) until a poppy plant of *Papaver somniferum* is obtained having an (S)-reticuline to phenanthrene alkaloid ratio of 100% or greater by weight.

Preferably in the aforementioned products and methods, the (S)-reticuline to phenanthrene alkaloid ratio is 200% or greater by weight, even more preferably the ratio is 1250% or greater and highly preferred is a ratio of about 2500%.

It is also highly preferred that there are substantially no phenanthrene alkaloids present.

The invention also consists in (S)-reticuline when obtained from any of the forgoing plants or plant products.

According to a sixteenth aspect there is provided a method for purifying reticuline from an aqueous extract of poppy straw comprising the following steps:

(i) mix said extract with toluene at near neutral pH and separate the aqueous and the non-aqueous phases,

(ii) mix aqueous phase from step (ii) with toluene at pH of about 8.5 to about 9.5 and separate the aqueous and the non-aqueous phases,

(iii) extract reticuline from the non-aqueous phase by caustic extraction.

Preferably the method further comprises the steps of (iv) mixing caustic extract of step (iii) with toluene at alkaline pH and separating the aqueous and the non-aqueous phases, (v) mixing the non-aqueous phase from step (iv) with water at acidic pH, and separating the aqueous and the non-aqueous phases, (vi) adding alkali to aqueous phase at ambient temperature, ageing for a time sufficient to induce formation of a precipitate and collecting precipitate containing reticuline.

Unless the context clearly requires otherwise, throughout the description and the claims, the words 'comprise', 'comprising', and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of "including, but not limited to".

Those skilled in the art will appreciate also that there are other methods of affecting the targeted enzymes to increase the accumulation of (S)-reticuline, such as transfection and targeting of genes and/or m-RNA encoding the production of (S)-reticuline oxidase, dihydroreticuline reductase and berberine bridge enzyme (BBE).

## BRIEF DESCRIPTION OF FIGURES

Figure 1 shows a HPLC trace of an extract of modified *Papaver somniferum* (bottom line) and an extract spiked with a standard for alkaloid analysis.

## DETAILED DESCRIPTION OF THE INVENTION

Utilizing the mutagenized plants of *Papaver somniferum* as described herein, persons skilled in the art easily know how to grow and reproduce such plants, collect the latex or the dried straw and purify the (S)-reticuline. As one embodiment of the present invention, seeds to the mutagenized plants of *Papaver somniferum*, as described herein,

2. Filter the resulting concentrate through a bed of Celite, and wash the bed with a cake volume of warm water.

Perform steps 3 to 17 at 40°C.

3. Add 0.3 volumes toluene, and adjust the pH to 6.8 using 40% w/v KOH or NaOH solution. Mix for 10 minutes, settle for 15 minutes. Separate the phases.

4. Perform a second toluene wash on the aqueous phase at pH 6.8, as in step 3.

5. Combine the toluene washes, and treat as spent.

6. Add 0.3 volumes of toluene to the aqueous phase, and adjust the pH to 9.3 using 40% w/v KOH solution. Mix for 10 minutes, settle for 15 minutes. Separate the phases.

7. Perform a second toluene extraction on the aqueous phase at pH 9.3, as in step 6.

8. Combine the toluene extracts, treat the aqueous phase as spent.

9. Add 0.3 volumes of a 2% w/v KOH solution to the toluene extracts. Mix for 10 minutes, settle for 15 minutes. Separate the phases.

10. Perform a second caustic extraction as in step 9, and combine the caustic extracts. Treat the toluene stream as spent solvent.

(To minimise the time that reticuline is kept in highly alkaline conditions, this caustic solution should not be stored for a long period, ie not more than 8 hours).

11. Add 0.5 volumes toluene to the caustic extracts, and adjust the pH to 9.3 using concentrated $H_3PO_4$. Mix for 10 minutes, settle for 15 minutes. Separate the phases.

12. Perform a second toluene extraction at pH 9.3, as in step 11.

13. Combine the toluene extracts, treat the aqueous phase as spent.

14. Add 0.3 volumes water to the toluene extracts, and adjust the pH to 4.5 using concentrated $H_3PO_4$. Mix for 10 minutes, settle for 15 minutes. Separate the phases.

15. Perform a second extraction at pH 4.5, as in step 14.

16. Combine the acid extracts, treat the toluene phase as spent.

17. Slowly add 8% v/v ammonia to the acid extract to adjust the pH to 9.3. Ensure that agitation is sufficient to dissolve any localised precipitation, and adjust ammonia addition accordingly.

18. Age for 4-8 hours at ambient, filter, wash cake with two cake volumes of water, and dry the solid.

19. Extract the mother liquors with toluene (2 x 0.2 volumes) at pH 4.5. This toluene extract should be recycled to a later batch, or extracted into an aqueous acid solution for precipitation at pH 9.2.

The results of the process are summarised in Table 2 below.

Table 2: HPLC assay results

| | | | | | Reticuline (3) | | |
|---|---|---|---|---|---|---|---|
| Step | pH | Sample | Colour of solution | Volume (ml or g) | g/L | grams | %age yield |
| 1 | 5.5 | filtered concentrate | dark | 1200 | 12.04 (1) | 14.45 | 100% |
| | | filtercake | green | 100 | 0.76 | 0.08 | 1% |
| 2 | 6.8 | toluene wash | green | 550 | 1.61 | 0.89 | 6% |
| | | conc after wash | dark | 1500 | 9.04 | 13.56 | 94% |
| 3 | 9.3 | spent aqueous | dark | 1550 | 0.45 | 0.70 | 5% |
| | | toluene extract | yellow | 500 | 25.00 | 12.50 | 87% |
| 4 | 13.5 | spent toluene | colourless | 500 | 0.01 | 0.01 | 0% |
| | | caustic extract | black | 450 | 27.54 | 12.39 | 86% |
| 5 | 9.3 | spent aqueous | black | 430 | 0.25 | 0.11 | 1% |
| | | toluene extract | colourless | 400 | NOT | ASSA | YED |
| 6 | 4.5 | spent toluene | colourless | 400 | 0.00 | 0.00 | 0% |
| | | acid extract | light yellow | 400 | NOT | ASSA | YED |
| 7 | 9.3 | dried solid | creamy yellow | 11.8 | 86.3% | 10.18 | 70% |
| | | mother liquors | yellow | 500 | 4.25 | 2.13 | 15% |
| | | dried 2nd crop solid (2) | yellow | 2.2 | 91.0% | 2.13 | 0.15 |

Note: 1) reticuline result for filtered concentrate based on combined results for step 2.

2) 2nd crop was isolated after extraction with toluene at pH9.2, and evaporation of the extract to dryness.

(3) Concentrations of reticuline were calculated using a laudanine standard.

Accurate quantitation of reticuline was not possible due to the lack of a reticuline standard. The results in Table 2 are relative to a laudanine standard purified locally.

Precipitation of the crystalline crude reticuline base at pH 9.2 was very difficult due to gum formation. It was necessary to add the ammonia very slowly to allow localised precipitation to dissolve, and gum formation was minimised by adding dilute ammonia (3 fold dilution with water to 8-10% w/w)

It was observed that the relatively pure aqueous solutions of reticuline were dark yellow at pH > 8, but light yellow in acidic conditions. A light-coloured acid solution of reticuline, therefore, gave rise to yellow coloured reticuline base solid.

The total quantity of crude reticuline (average assay 80%) obtained from all of the available straw was 21.5 grams as dry weight.

The process summarised in Scheme 4 (described in detail in Scheme 5) represents a good method for the isolation of (S)-reticuline rich extracted alkaloid mixture from poppy straw. Implementation of this process on a large scale may require some minor alterations, such as the use of lime to treat the straw instead of acetic acid to reduce metal corrosion. This process could be scaled up to a factory with no specialised apparatus being necessary for the large scale extraction of reticuline.

This process is sufficient to produce (S)-reticuline product of at least 80% purity. Further purification may be accomplished by use a co-solvent during precipitation or isolating a salt of reticuline, such as the bitartrate or the oxalate.

The procedure described in Scheme 5 does not represent any major hazards other than those that currently exist in the morphine extraction process. No excessive temperatures or unusual solvents or reagents are required.

Although the invention has been described with reference to specific embodiments, modifications that are within the knowledge of those skilled in the art are also contemplated as being within the scope of the present invention.

416 Rec'd PCT/PTO 15 JUL 2000

SERIAL NO. _____ DOCKET NO. JJ1763 BY DH/SH

APPLICATION OF Fist et al MAILED: 7-14-00

ENTITLED IMPROVED PRODUCTION OF RETICULINE

09/600500

THE FOLLOWING HAS BEEN RECEIVED IN THE U.S. PATENT OFFICE ON THE DATE STAMPED HEREON:

- ☐ AFFIDAVIT
- ☒ DECLARATION Unexecuted
- ☒ AMENDMENT Preliminary
- ☐ ASSIGNMENT FOR RECORDING
- ☐ BRIEF
- ☒ CHARGE TO DEPOSIT ACCOUNT 10-0750
- ☒ DRAWINGS 1
- ☐ ISSUE FEE TRANSMITTAL
- ☒ LETTER Transmittal PTO-1390
- ☐ PCT FILING
- ☐ IDS - FORM-1449
- ☐ EXTENSION OF TIME
- ☐ NOTICE OF APPEAL
- ☐ OATH OR ☐ DECLARATION
- ☐ POWER OF ATTORNEY
- ☐ PRELIMINARY STATEMENT
- ☐ PRIORITY DOCUMENT
- ☐ STATUS INQUIRY
- ☒ SPECIFICATION 32 PGS
- ☒ CLAIMS 60
- ☐ _____

FORM 113788 REV. 7/96

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